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Animal models of arthritis in NOS2-deficient mice

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Summary

Objective: To study the role of nitric oxide (NO) in cartilage destruction in murine models of arthritis and osteoarthritis.

Methods: Joint inflammation was induced in the knee joint by intraarticular injection of Zymosan. Osteoarthritis was induced by local injection of bacterial collagenase, causing joint instability. The effect of NO deficiency was studied by comparing the effects in normal mice and mice with genetically disrupted NOS2 (inducible NO synthase). Impact on articular cartilage was evaluated by histology and measurement of chondrocyte 35S-proteoglycan synthesis.

Results: NOS2 deficiency prevented chondrocyte proteoglycan synthesis inhibition in the arthritic cartilage and restored normal responsiveness to IGF-1. Net cartilage proteoglycan depletion was markedly reduced in the absence of NOS2, although inflammation was hardly affected. Osteoarthritic joint pathology was also significantly reduced, including diminished cartilage lesions and osteophyte formation.

Conclusion: NO plays a major role in cartilage damage in both arthritic and osteoarthritic conditions.

Key words: NO, Arthritis, Osteoarthritis.

Introduction

Chronic joint inflammation is characterized by cartilage destruction, which consists of inhibited synthesis of matrix as well as enhanced breakdown of matrix. It is found that IL-1 plays a major part in this destruction, predominantly through its key role in the process of proteoglycan synthesis inhibition.[1] Although IL-1 can be involved in proteoglycan breakdown, substantial overkill by other mediators may occur. Although inhibition of IL-1 does not always prevent early proteoglycan loss in acute inflammation, it reduces late, erosive changes.[2] Apart from the impact of IL-1, disturbed chondrocyte matrix synthesis may result from a disturbance in proper IGF-1 signaling.[3]

IL-1 is a potent stimulant of nitric oxide (NO). Cells in the synovial tissue and articular chondrocytes can produce considerable amounts of NO and the production in arthritic conditions can be linked to constitutive NO-synthase as well as inducible NO-synthase (iNOS or NOS2). Studies with inhibitors of NO have suggested that NO plays a major role in IL-1 effects and joint pathology. However, the inhibitors used are not fully selective and results may be flawed by side effects. In the present study we made use of NOS2 deficient mice[4] to study its role in arthritis and osteoarthritis. Since immune driven inflammation can be markedly impaired in NOS2 deficient mice, we confined the study to the model of nonimmune inflammation, induced with Zymosan. For comparison, we also studied the impact of deficiency of the cytokines TNF, IL-1b, and IL-6. Moreover, the OA model was studied in NOS2 and IL-1b knock-out mice.

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Materials and methods

ARTHRITIS MODEL

A dose of 180 ug Zymosan was injected into the right knee of the various mouse strains. At various days thereafter, patellar cartilage was isolated and cultured for 3 hours in the presence of 35S-sulfate, or first incubated for 24 hours in a medium with or without IGF-1 (0.5 ug/ml), before 35S-sulfate pulse labeling. At the end of the experiment whole knee joints were isolated, processed for histology and stained with Safranin O to depict proteoglycan loss in the cartilage.

OSTEOARTHRITIS MODEL

1 U bacterial collagenase was injected into the knee joint at days 1 and 4. This caused minor joint inflammation and no measurable cartilage damage in the acute stage. At day 42 the mice were killed and knee joints processed for histology. Cartilage lesions were evaluated in the tibial-femoral area and osteophytes were scored at the margins of tibia and femur and at insertion sites of ligaments.

Results and discussion

PROTEOGLYCAN SYNTHESIS

Joint inflammation induced with Zymosan does result in marked inhibition of proteoglycan synthesis in the articular cartilage. This was evident at day 1 after induction of inflammation and synthesis remained suppressed as long as there was active inflammation. When joint inflammation was induced in NOS2 deficient mice, proteoglycan synthesis was not reduced. In fact, the synthetic activity was even above normal at day 2 (Fig. 1a). For comparison,

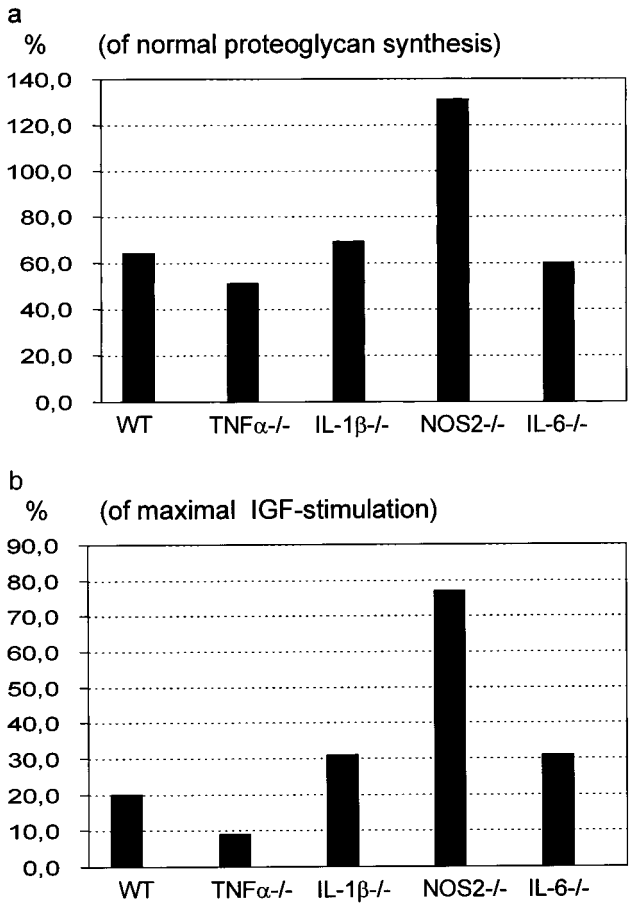

Fig. 1. A: Proteoglycan synthesis in patellar cartilage measured at day 2 after induction of Zymosan arthritis, by 3 h pulse labeling with 35S-sulfate *in vitro*. Synthesis is expressed as a percentage of the activity in normal cartilage. B: The above cartilage specimens are cultured for 24 h in the presence or absence of IGF-1, followed by 3 h 35S-sulfate labeling. Values are expressed as a percentage of IGF stimulation compared to the respective controls in the absence of IGF-1. The stimulation found in normal cartilage is posed at 100%.

similar studies performed in other knock-out mice revealed that TNF-α or IL-6 deficiency had no major influence on this parameter. Moreover, IL-1b deficiency did not reduce this suppression. Preliminary studies with additional treatment of the latter mice with anti-IL-1α antibodies made it clear that both types of IL-1 have to be suppressed, before full restoration of PG synthesis can be accomplished. It further demonstrates that IL-1α is a prominent isoform in early arthritis. The negative findings in the TNF-α and IL-6 deficient mice underline two key points: TNF-α is not a dominant inducer of IL-1 in this model, although such a role has been claimed in rheumatoid arthritis; moreover, IL-6 is not a secundary cytokine, mediating the IL-1 effects. In fact, studies at later stages of the Zymosan model made it clear that net proteoglycan loss is enhanced in IL-6 deficient mice, implying a protective role of IL-6 in destruction.[5]

IGF-RESPONSIVENESS

Apart from direct ex-vivo proteoglycan synthesis measurements in the arthritic cartilage, we also analysed the IGF responsiveness in a subsequent 24 h culture period of the isolated patellar cartilage specimens. Previous studies showed that arthritic chondrocytes are non-responsive to the main anabolic stimulus IGF-1.[3] Now we show that NO is a crucial element in this phenomenon. Figure 1b displays that cartilage from wild type, arthritic mice only shows 20% stimulation of its PG synthesis in the presence of IGF-1, as compared to a 100% stimulation in normal cartilage. This IGF-1 stimulation pattern is not significantly different in arthritic cartilage obtained from TNF-α, IL-1β or IL-6 knock-outs, whereas the IGF-1 response was nearly normal in the NOS2 deficient cartilage specimens.

INFLAMMATION

Acute joint inflammation was not significantly reduced in the IL-1b and NOS2 deficient mice,[6] marked reduction in joint swelling was seen in the TNF-α knock-out, whereas inflammation was also reduced in the IL-6 deficient animals.

CARTILAGE PROTEOGLYCAN DEPLETION

This was measured by loss of Safranin O staining of histologic joint sections. At day 7 cartilage proteoglycan depletion was more than 50% reduced in the NOS2 deficient mice as compared to the loss in wild type mice. Intriguingly, progressive loss of proteoglycan was seen at days 14 and 21, both in the wild type and in the NOS2 deficient mice, in spite of the fully normalized synthetic activity in the latter, and the relative differences remained similar. This implies that the early differences were due to the normalization of synthesis, but that proteolytic breakdown is on ongoing process in the joint inflammation, apparently independent of NO. This is in line with earlier in vitro studies which revealed that IL-1 mediated inhibition of cartilage proteoglycan synthesis was NO dependent,[7] but that IL-1 mediated cartilage degradation was similar or even enhanced in the presence of NO inhibitors.

For comparison, late histology in IL-1β and TNF deficient mice shows almost complete protection from PG depletion in the IL-1β −/− mice, whereas there was still considerable PG depletion in the absence of TNF-α.

OA PATHOLOGY IN NOS2 DEFICIENT MICE

Osteoarthritis was induced in normal and NOS2 deficient mice by local injection of bacterial collagenase into the knee joint. Cartilage lesions develop after a few weeks in the weight bearing sites in the tibial plateau, in line with the biomechanical nature of the model.[8] In fact, collagenase damages the ligament structures of the joint and provokes major instability. Osteophytes occur before cartilage lesions. Figure 2 shows the comparison of the pathology in normal and NOS2 deficient mice at day 42. It is clear that both the degree of cartilage damage as well as the extent of osteophyte formation is reduced in the absence of inducible NO. Recent studies in the canine ACL model revealed similar suppressive effects with NO inhibitors.[9] Moreover, blocking of IL-1 also provided reduction of lesions in the canine model.[10] Our preliminary observations in the IL-1β deficient mice suggested that cartilage damage was reduced when minor instability was induced, but when the model was made more severe the protective effect in the absence of IL-1β was lost. This might suggest that the

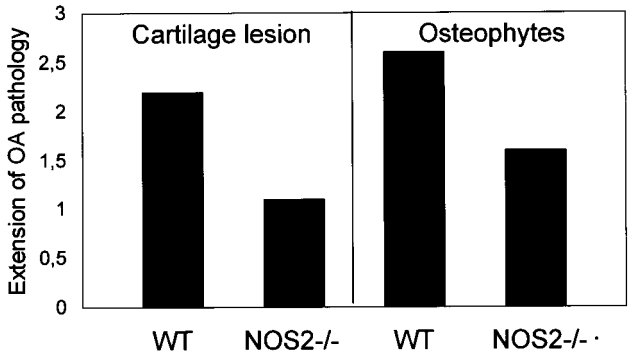

Fig. 2. OA cartilage lesions in the tibial-femural area and osteophyte formation at the joint margins at day 42 after induction of collagenase instability OA. Values are the mean of groups of 15 mice.

protective effect of NO inhibition in OA is independent of IL-1. In fact, proteoglycan synthesis is enhanced in stead of inhibited in early OA, not compatible with a dominant role of IL-1 in that stage. In late stages of OA, chondrocyte proteoglycan synthesis does diminish and a more dominant role of IL-1 seems likely. There is no doubt that NO production is enhanced in late stage human OA and IL-17 appears a probable inducer.[11]

Apart from cartilage lesions, we also analyzed the osteophytes. These were 50% reduced as well in the NOS2 deficient mice (Fig. 2). The mechanism of osteophyte formation is not fully understood, but it is clear that TGF-β can induce osteophytes which resemble the OA osteophytes, making this growth factor a likely candidate.[12,13] We recently injected or overexpressed TGF-β in the knee joint of NOS2 deficient mice, but could not find reduced osteophyte formation in these animals. This implies that the TGF-β effect is not NO dependent, leaving reduced TGF-β production in NO deficient mice as a potential mechanism.

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